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Supplemental information

Antigen spacing on protein nanoparticles influences antibody responses to vaccination

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SUPPLEMENTARY FIGURES

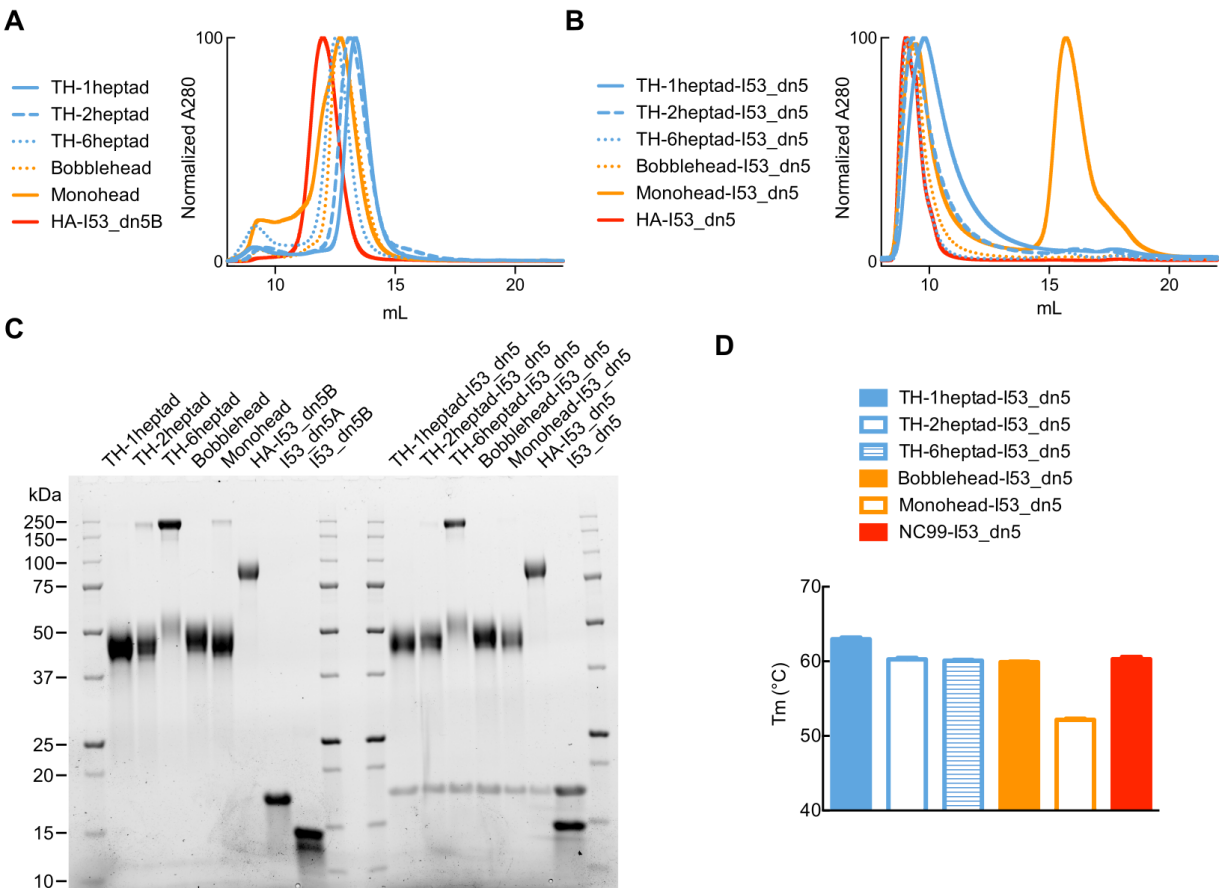

Figure S1 Purification of trihead nanoparticle extension series, Related to Figure 2.

(A) SEC chromatogram of trihead extension series components on a Superdex 200 Increase 10/300 GL column.

(B) SEC chromatogram of trihead extension series nanoparticles on a Superose 6 Increase 10/300 GL column.

(C) SDS-PAGE of trihead extension series components and nanoparticles.

(D) Melting temperatures of trihead extension series nanoparticles as measured by NanoDSF. The bars and errors bars represent the mean ± SEM for technical replicates (n = 3).

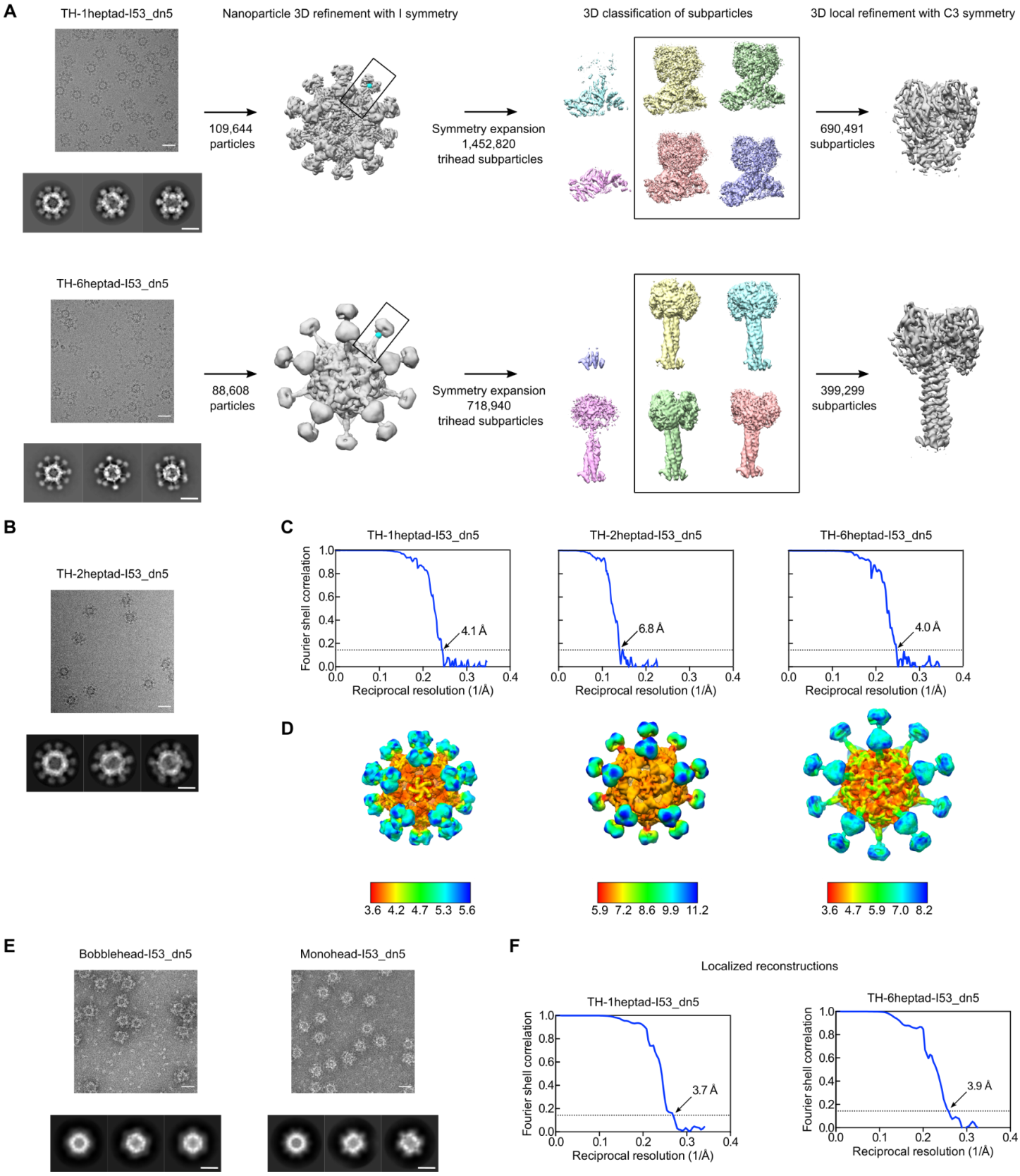

Figure S2 Negative Stain and Cryo-EM Data Processing, Related to Figure 3.

(A) Trihead reconstruction cryo-EM processing diagram for TH-1heptad-I53_dn5 (top) and TH-6heptad-I53_dn5 (bottom). Representative cryo-EM micrographs, scale bar = 50 nm, and 2D class averages, scale bar = 25 nm, and 2D class averages (left). Trihead nanoparticle reconstructions with marker (shown here in cyan) used to define the location of

trihead trimers within the icosahedral symmetry of the nanoparticles and to extract subparticles, which were then subject to 3D classification (middle). Boxed out 3D classes were combined and used in final local refinements (right).
(B) Representative cryo-EM micrograph and 2D class averages for TH-2heptad-I53_dn5.
(C) Gold-standard Fourier shell correlation (FSC) curves for the trihead nanoparticle reconstructions in Figure 3A. The 0.143 cutoff is indicated by the dashed lines.
(D) Local resolution maps of the trihead nanoparticle reconstructions.
(E) Representative nsEM micrographs, scale bar = 50 nm, and 2D class averages, scale bar = 25 nm, of bobblehead and monohead nanoparticles.
(F) Gold-standard FSC curves for TH-1heptad-I53_dn5 and TH-6heptad-I53_dn5 localized reconstructions in Figure 3B.

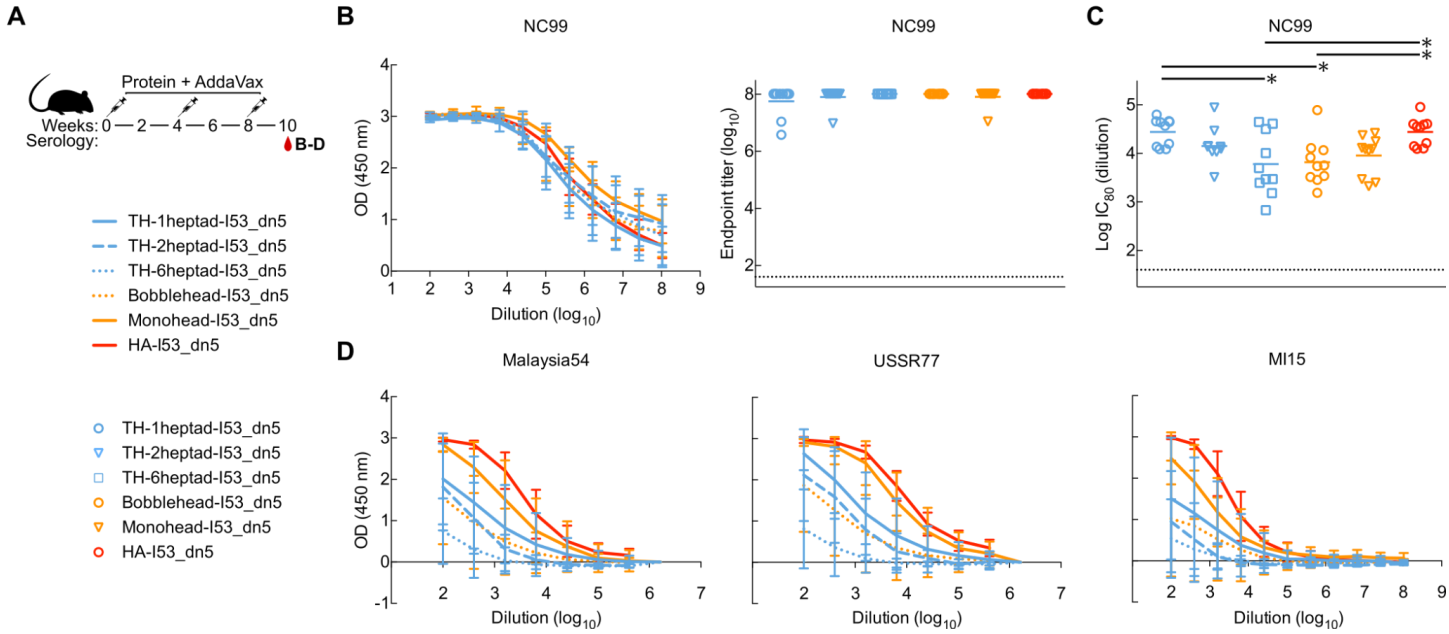

Figure S3 Immune Responses in Mice Immunized with Trihead Nanoparticle Extension Series, Related to Figure 4.

(A) Mouse immunization schedule and groups for adjuvanted trihead nanoparticle extension series.

(B) Vaccine-matched NC99 ELISA curves and binding titers in adjuvanted immune sera.

(C) Vaccine-matched NC99 ELISA microneutralization titers in adjuvanted immune sera.

(D) Adjuvanted immune sera ELISA curves against vaccine-mismatched H1 foldon trimers.

Each symbol represents an individual animal, and the geometric mean of each group is indicated by the bar (n = 9-10 mice/group). Each line and error bar represents the geometric mean and geometric mean SD, respectively, of the absorbance at 450 nm (n = 9-10 mice/group). Statistical significance was determined using one-way ANOVA with Tukey's multiple comparisons test; ∗p < 0.05; ∗∗p < 0.01; ∗∗∗p < 0.001; ∗∗∗∗p < 0.0001.

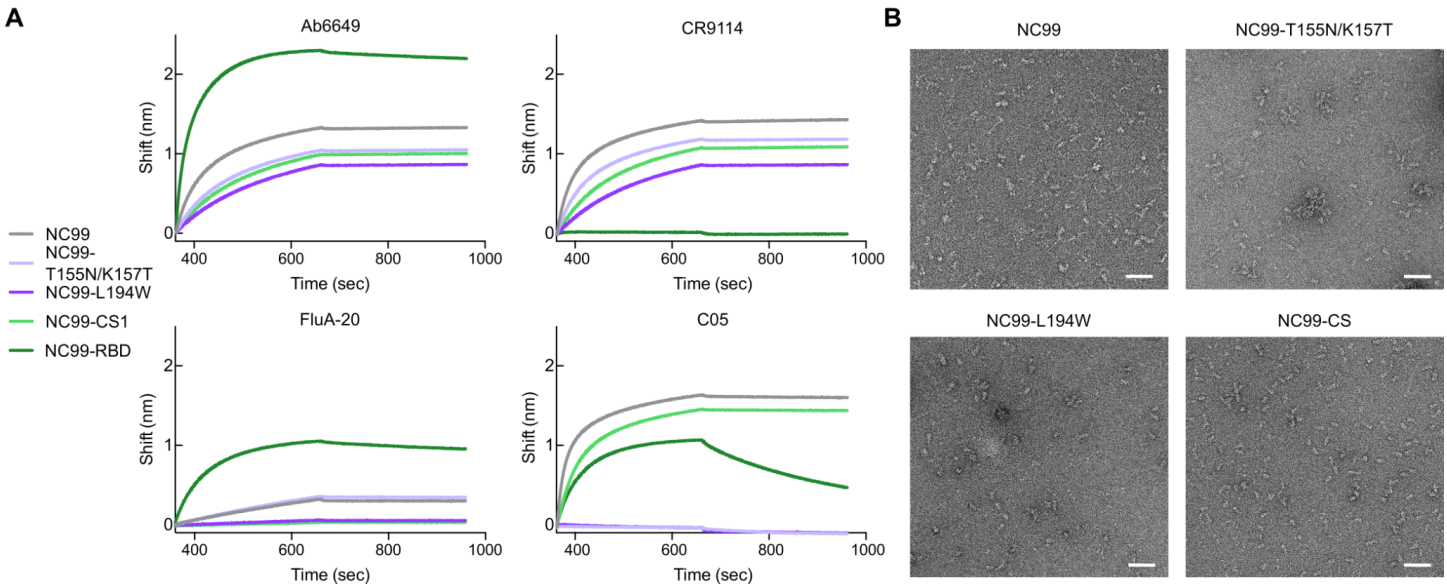

Figure S4 Biophysical characterization of NC99 probes, Related to Figure 5.

(A) BLI of NC99 probes against various HA mAbs.

(B) nsEM micrographs of NC99 probes. Scale bars = 50 nm.

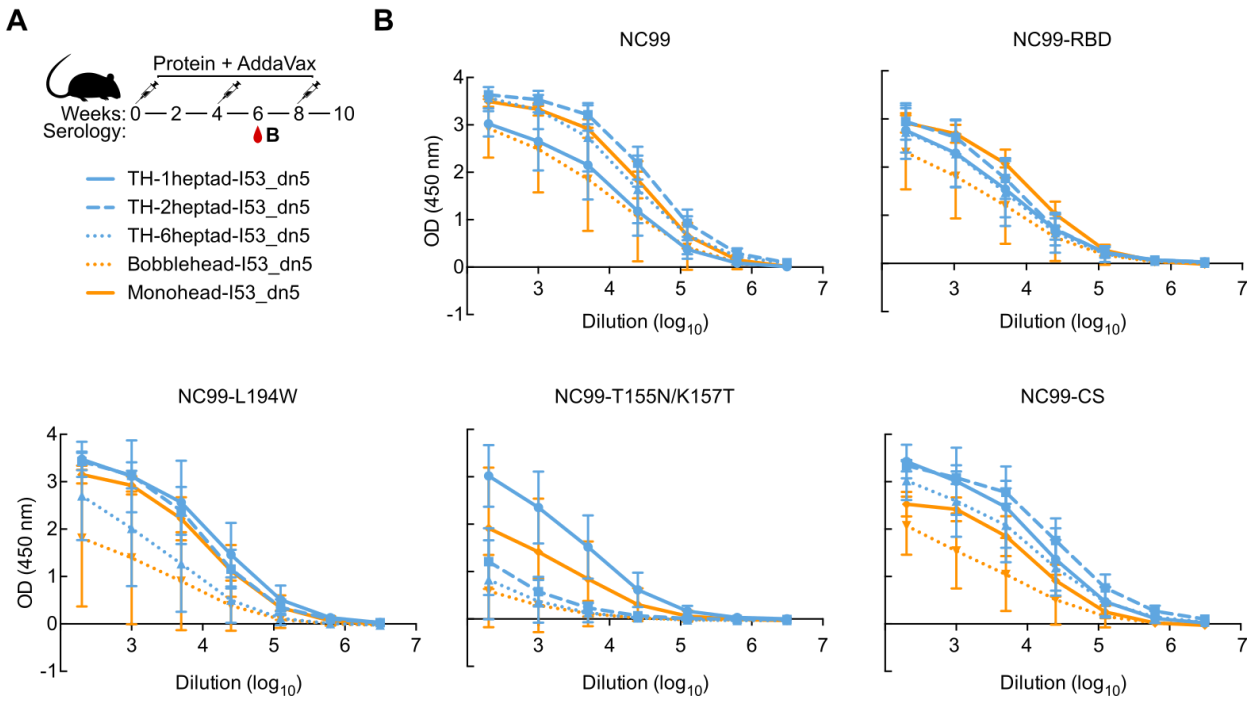

Figure S5 Epitope Mapping of Vaccine-elicited Antibodies, Related to Figure 5.

(A) Mouse immunization schedule and groups for adjuvanted trihead nanoparticle extension series.

(B) Week 6 adjuvanted immune sera ELISA curves against various NC99 probes. Each line and error bar represents the geometric mean and geometric mean SD, respectively, of the absorbance at 450 nm (n = 9-10 mice/group).

Table S1. Amino acid sequences of proteins used in this study, Related to STAR Methods.

>Monohead
MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADY
EELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGF
GSGSGKRIENILSKIYHIENEIAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALE
LDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>Monohead-SS1
MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADY
EELRCQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGF
GSGSGSCIENINSKIYHIENEIAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYNLGNAYYKQGRYREAIEYYQKAL
ELDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>TH-1heptad
MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADY
EELRCQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVLVVSSHYDRVFTPIIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFG
SGSGSCIEHIENEIAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALELDPNNAE
AWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>TH-2heptad
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EELRCQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVLVVSSHYDRVFTPIIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFG
SGSGSCIENINSKIYHIENEIAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYNLGNAYYKQGRYREAIEYYQKALEL
DPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHHHH

>TH-6heptad
MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADY
EELRCQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVLVVSSHYDRVFTPIIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFG
SGSGSCIENINSKIYHIEDKIEEINRKIEHILSKIYHIERKIEEILNEIAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYN
LGNAYYKQGRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGG
WELQHHHHHH

>Bobblehead-I53_dn5
MDSKGSSQKGSRLLLLLVVSNLLLPQGVLAIAPLQLGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADY
EELRCQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVLVVSSHYDRVFTPIIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGFG
SGSGSCIENINSKIYHIENEIARIKKLIGESGGSGGESAELAYLLGELAYKLGEYRIAIRAYRIALKSDPNNAEAWYNLGNAYYK
QGRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHH
HHH

>HA-I53_dn5
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CELLISKESWSYIVETPNPENGTCFPGYFADYEELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNL
LWLTGKNGLYPNLSKSYVNNKEKEVLVLWGVHHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINY
YWTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSAKL
RMVTGLRNIPSIQSRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQFTA
VGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCNN

ECMESVKNGTYDYPKYSEESKLNREKIDGVSAEEAELAYLLGELAYKLGEYRIAIRAYRIALKRDPNNAEAWYNLGNAYYKQ
GRYREAIEYYQKALELDPNNAEAWYNLGNAYYERGEYEEAIEYYRKALRLDPNNADAMQNLLNAKMREEGGWELQHHHH
HH

>I53_dn5A.1
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TPHFDYIADSTTHQLMKLNFELGIPVIFGVITADTDEQAEARAGLIEGKMHNHGEDWGAAAVEMATKFNLEHHHHHH

>NC99
MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLEDSHNGKLCLLKGIAPLQLGNCSVAGWILGNPE
CELLISKESWSYIVETPNPENGTCFPGYFADYEELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNL
LWLTGKNGLYPNLSKSYVNNKEKEVLVLWGVHHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINY
YWTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSAKL
RMVTGLRNIPQRETRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQFTA
VGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCNN
ECMESVKNGTYDYPKYSEESKLNREKIDGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHH
H

>NC99-T155N/K157T
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LWLNGTNGLYPNLSKSYVNNKEKEVLVLWGVHHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINY
YWTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSAKL
RMVTGLRNIPQRETRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQFTA
VGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCNN
ECMESVKNGTYDYPKYSEESKLNREKIDGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHH
H

>NC99-L194W
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LWLTGKNGLYPNLSKSYVNNKEKEVLVLWGVHHPPNIGNQRAWYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRIN
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KLRMVTGLRNIPQRETRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQF
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NNECMESVKNGTYDYPKYSEESKLNREKIDGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHH
HHH

>NC99-CS
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CELLISKESWSYIVETPNPENGTCFPGYFADYEELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNL
LWLTGKNGLYPNLSKSYVNNKEKEVLVLWGVHHPPNIGNQRALYHTENAYVLVVSSHYDRVFTPIIAKRPKVRDQEGRINYY
WTLLEPGDTIIFEANGNLIAPWYAFALSRGFGSGIITSNAPMDECDAKCQTPQGAINSSLPFQNVHPVTIGECPKYVRSAKLR
MVTGLRNIPQRETRGLFGAIAGFIEGGWTGMVDGWYGYHHQNEQGSGYAADQKSTQNAINGITNKVNSVIEKMNTQFTAV
GKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLENERTLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFEFYHKCNNE
CMESVKNGTYDYPKYSEESKLNREKIDGSGYIPEAPRDGQAYVRKDGEWVLLSTFLGSGLNDIFEAQKIEWHEGHHHHHH

>NC99-RBD
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EELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
HHPPNIGNQRALYHTENAYVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYYWTLLEPGDTIIFEANGNLIAPWYAFALSRGA
GSGLNDIFEAQKIEWHELEVLFQGPGHHHHHH

>NC99-RBD-TI
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EELREQLSSVSSFERFEIFPKESSWPNHTVTGVSASCSHNGKSSFYRNLLWLTGKNGLYPNLSKSYVNNKEKEVLVLWGV
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SGLNDIFEAQKIEWHELEVLFQGPGHHHHHH

Table S2. Cryo-EM data collection and refinement statistics, Related to Figure 3.

	TH-1heptad-I53_dn5 EMD: 42481	TH-1heptad-I53_dn5 (local refinement) PDB: 8UR5 EMD: 42482	TH-2heptad-I53_dn5 EMD: 42483	TH-6heptad-I53_dn5 EMD: 42485	TH-6heptad-I53_dn5 (local refinement) PDB: 8UR7 EMD: 42486
Data collection and processing					
Magnification (×)	36,000	36,000	130,000	36,000	36,000
Voltage (kV)	200	200	300	200	200
Electron exposure (e^{-}/Å^2)	60	60	70	60	60
Defocus range (μm)	-0.5 - -2.5	-0.5 - -2.5	-0.5 - -2.5	-0.5 - -2.5	-0.5 - -3.5
Pixel size (Å)	1.16	1.16	0.525	1.16	1.16
Symmetry imposed	I	C3	I	I	C3
Final particle images (no.)	72,641	690,491	1,986	35,947	399,299
Map resolution (Å)	4.1	3.7	6.8	4.0	3.9
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map sharpening *B* factor (Å^2)	-215	-244	-797	-186	-213
Validation					
MolProbity score		1.86			1.74
Clashscore		6.45			7.52
Poor rotamers (%)		1.71			0.66
Ramachandran plot					
Favored (%)		95.22			95.27
Allowed (%)		4.35			4.73
Disallowed (%)		0.43			0